

DNA MICROCON CONCENTRATION

A. SCOPE

Microcon centrifugal filter devices concentrate and desalt macromolecular solutions of 10-500 µL using a centrifuge. The anisotropic, hydrophilic Ultracel YM membranes in microcon centrifugal filter devices are characterized by a nominal molecular weight limit (NMWL), i.e., the ability to retain molecules above a specified molecular weight. The microcon centrifugal filter device used in this laboratory has an Ultracel YM-100 membrane with the ability to retain molecules above 100,000 NMWL. Microcons are utilized when the concentration of a neat sample is too low to allow for optimal amplification.

B. QUALITY CONTROL

- B.1 Protective gloves, a lab coat, and a mask must be worn at all times when performing this procedure to prevent contamination.
- B.2 Each new lot of microcons must undergo quality control testing prior to concentrating casework samples:

A portion of a non-probative sample extract and a water / TE⁻⁴ sample will undergo concentration using microcon centrifugal filter devices. The concentrated samples and the corresponding non-concentrated extract will be quantitated to confirm that the lot being tested is concentrating as expected and is free from contaminants. It is expected that the % concordance between the theoretical and the obtained quantitation results will be equal to or greater than 70% for the autosomal and Y chromosomal results.

Example:

[Non-concentrated Sample 1]: 0.0795ng/µL

Theoretical concentration of Sample 1 (adding 70µl to microcon and having 35µl remaining): $70 \times .0795 / 35 = 0.159 \text{ ng/}\mu\text{L}$ ((ul added to microcon)* ([non-concentrated])/(volume remaining post concentration))

Obtained concentration of Sample 1: 0.151ng/µL

% concordance between the [obtained] and the [theoretical] of Sample 1:
 $0.151 / 0.159 \times 100 = 94.97\%$

The quality control data will be placed into the critical reagent binder.

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C. SAFETY

- C.1 Protective gloves, a lab coat, a mask, and eye protection (e.g. safety glasses or a face shield) must be worn at all times when performing this procedure.
- C.2 Distinguish all waste as general, biohazard, or sharps and discard appropriately.

D. REAGENTS, STANDARDS, AND CONTROLS

- D.1 Bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner (Decontamination)

E. EQUIPMENT & SUPPLIES

E.1 Equipment

- E.1.1 Microcon centrifugal filter devices
- E.1.2 Microcentrifuge
- E.1.3 Pipettes

E.2 Supplies

- E.2.1 Kimwipes
- E.2.2 Microcentrifuge tubes
- E.2.3 Sterile aerosol resistant pipette tips
- E.2.4 Microcentrifuge tube racks
- E.2.5 Mask
- E.2.6 Lab coat
- E.2.7 Eye protection (e.g. safety glasses, face shield)
- E.2.8 Disposable gloves
- E.2.9 Permanent marker

F. PROCEDURE

- F.1 Label each assembled microcon unit.
- F.2 Add the entire (0.5 mL capacity) extracted DNA sample to the upper microcon reservoir. Close the cap.
- F.3 Spin in a microcentrifuge for 5 minutes at approximately 500 rcf.
- F.4 Discard the filtrate in the lower reservoir.
- F.5 Collect the concentrated DNA by inverting the upper reservoir into the labeled

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microcentrifuge tube provided or any other tube that fits the upper reservoir; the QIAcube elution tubes are not compatible with the upper reservoir. Leave the cap open and spin for 5 minutes at approximately 500 rcf.

- F.6 Repeat as necessary to complete concentration. Alternatively, it is acceptable to centrifuge a sample for longer in step F.3., for example, 10 minutes instead of repeating the procedure twice. Step F.5. will remain the same.
- F.7 Analysts may manually calculate or use an appropriate excel sheet (DOC ID [4128](#)) to calculate the approximate amount of DNA in post microcon concentration samples based on the quantification results that were obtained for the sample prior to concentration. All excel calculations will be checked by the analyst and during technical review.

G. INTERPRETATION GUIDELINES

Not applicable

H. REFERENCES

- H.1 Microcon Centrifugal Filter Device, User Guide.

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